J P H Burt *et al* 1989 *J. Phys. E: Sci. Instrum.* **22** 952-957

An optical dielectrophoresis spectrometer for low-frequency measurements on colloidal suspensions

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Print publication: Issue 11 (November 1989)

Abstract. An optical system is described for measuring the response of suspended particles to imposed non-uniform electric fields in the frequency range 1 Hz to 4 MHz. Such dielectrophoretic measurements can provide details of the dielectric and surface charge properties of animate and inanimate particles. This simple, low-cost and rapid technique extends these measurements down to lower frequencies than previously reported, and has revealed new effects associated with particle surface charge and surface conductivity. At the lower frequencies corrections are required to take account of the electrode polarisation effects, which modify the electric field distribution in the colloidal suspension between the electrodes. Measurements are reported for silicon powder, yeast and the bacteria Micrococcus lysodeikticus and are compared with theoretical expectations. Biotechnological applications include cell separation, cell characterisation, cell-culture quality control and biomass determination.

doi:10.1088/0022-3735/22/11/011
URL: http://stacks.iop.org/0022-3735/22/952

Biochimica et Biophysica Acta, 1034 (1990) 93–101
Elsevier

BBAGEN 23280

Dielectrophoretic characterisation of Friend murine erythroleukaemic cells as a measure of induced differentiation

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(Received 12 September 1989)

Key words: Dielectrophoresis; Cell surface; Membrane conductivity; Surface charge; (Friend murine erythroleukemia cell); (Human red blood cell)

Dielectrophoresis measurements, the study of the motion of particles in non-uniform a.c. electrical fields, have been made on three cell lines (DS19, R1 and DR1) of Friend murine erythroleukaemia cells as a function of hexamethylene bisacetamide (HMBA) treatment. The effects of saponin treatment on R1 cells and neuraminidase on human red blood cells were also studied. It is shown that the dielectrophoretic behaviour can be interpreted in terms of cell surface charge and cell membrane conductivity effects. HMBA reduces the cell surface charge on all three cell lines, and in lines DS19 and DR10, where the cells are induced to differentiate, there is an increase in effective cell conductivity. This gain in conductivity is concluded to be associated with either an enhanced lateral electrophoretic motion of delocalised ions or of the polarisability of dipoles at the membrane surface.

Introduction

Dielectrophoresis is defined as the motion of electrically charged or neutral particles induced by non-uniform a.c. or d.c. electric fields [1]. This phenomenon has been applied to a variety of practical applications such as the separation and levitation of cells and particles [1–5] as well as for the investigation of the dielectric properties of biological and non-biological materials [6–9].

The general phenomenological equation describing the dielectrophoretic force, F, acting on a particle of volume, v, in a non-uniform electric field is given by

$$F = pv(E \cdot \nabla)E \quad (1)$$

where p is the effective polarisability of the suspended particle, E is the local root-mean-square electric field and ∇ is the del vector operator. Since the polarisability parameter p is frequency dependent, then the force F will also vary with frequency. This study is primarily concerned with the characterisation of this frequency dependence.

An optical measurement system has been described [10] for obtaining dielectrophoretic spectra in the frequency range 1 Hz to 4 MHz for particles of diameter from 1–40 μm. This provides more rapid data collection than the microscopy-based technique developed by Pohl [1], which uses time-lapse photography to determine the rate of collection of the test particles at the electrodes used to generate the non-uniform electric field. Using the new technique the dielectrophoretic properties of cells have been investigated for frequencies down to 1 Hz where membrane surface charge effects dominate the overall dielectrophoretic response [10,11]. In the mid-range of frequencies extending from around 200 Hz to 2 kHz the response appears to be controlled by the effective conductivity of the cells, whereas for frequencies higher than this, the effective dielectric permittivity of the cell becomes the dominant influencing factor. Using this method, therefore, the dielectrophoretic characteristics of cells can be used to investigate cell surface and cell membrane properties.

The bioelectrical properties of the membranes of neoplastic mammalian cells have frequently been observed to differ from those of comparable cells of normal phenotype, and it has been suggested that such altered properties may be related to the genesis and maintenance of the malignant state [12–16]. Here we

Abbreviations: HMBA, hexamethylene bisacetamide; DMSO, dimethylsulphoxide.

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have applied the technique of dielectrophoresis to the study of three Friend murine erythroleukaemia (MEL) cell lines as a function of treatment with agents that induce these cells to differentiate. These well characterised cell lines have been widely investigated as models of the sequences that lead to terminal cell differentiation [17].

The results demonstrate that dielectrophoresis can reveal significant alterations in the bioelectrical properties of these cell models during treatment with differentiating agents. Furthermore, these studies provide confirmation that dielectrophoretic measurements can distinguish between cell surface charge and cell membrane conductivity characteristics.

Materials and Methods

Friend murine erythroleukaemia (MEL) clones R1, DS19 and DR10 were seeded in suspension cultures in 300 ml of medium, supplemented with 10% foetal calf serum and 1 mM glutamine, and gassed with 5% CO_2/air mixture. The cultures were seeded at a density of 10^5 cells/ml in Gibco minimum essential medium containing 1 μg/ml gentamycin. Cells were passaged every 2 or 3 days so that cell density was always kept below 10^6/ml. Cells were harvested by centrifugation ($600 \times g$ for 7 min) and were counted using a haemocytometer. Cell viabilities were determined by Trypan blue dye exclusion.

To examine the effects of agents that induce differentiation, cells were seeded in culture media supplemented with 4 mM hexamethylene bisacetamide (HMBA) (Sigma) or 1% dimethylsulphoxide (DMSO) (Sigma) and grown for up to 4 days without refeeding. Parallel cultures grown under identical conditions, except for the absence of inducing agents, served as controls in all experiments.

In preliminary experiments on a variety of different mammalian cell types, problems were occasionally encountered with the maintenance of cell integrity in the low ionic strength dielectrophoresis media. Three major factors were identified which allowed this problem to be overcome in all of the cell lines that we have so far examined. The first factor was the choice of 320 mM sucrose solution as the cell suspending medium. This medium, which was determined to be 28% more hypertonic than 150 mM NaCl, prevented cell swelling and was far more satisfactory than a comparable mannitol solution which was often found to damage the cells. The use of a 320 mM sucrose solution also has a precedent as this medium is an accepted perfusion fluid that maintains adequate cell viability both in vitro and in vivo. Secondly, it was found that cells were very sensitive to glucose deprivation when suspended in low ionic strength solutions. This problem was circumvented by ensuring that all solutions contained 3 mg/ml glucose.

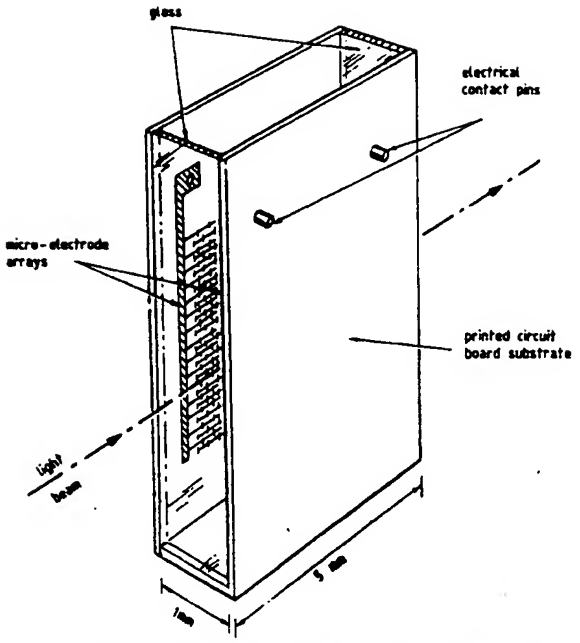

Fig. 1. Design of optical sample chamber and micro-electrodes.

Thirdly, the role of divalent cations in maintaining cell membrane integrity is well known and their presence has been proposed as essential for cells to overcome membrane stresses [18,19]. For erythrocytes that had been harvested in the presence of EDTA to prevent clotting, the addition of 1 μM $CaCl_2$ to the sucrose solutions greatly aided the maintenance of cell integrity without significantly increasing the electrical conductivity of the final cell suspensions.

Harvested cells were washed three times in the 320 mM sucrose plus 3 mg/ml glucose solution made with double-distilled, triple-deionised, water and the subsequent cell survival rates were determined to lie in the range 89–94%. After the final wash, cells were resuspended in this same solution, which served as the suspending medium for the dielectrophoretic measurements, to a cell concentration that gave an optical absorbance of 0.26 at 635 nm for a 1 cm pathlength. The conductivity of the cell suspensions, determined using a conductivity bridge (Radiometer type CDM2f, Copenhagen), was less than 650 μS/m in all experiments, and this enabled the dielectrophoretic measurements to be performed down to 1 Hz [10,11].

To measure the dielectrophoretic properties of the cells, 0.2 ml of cell suspension was pipetted into the optical chamber shown in Fig. 1 and subjected to the non-uniform electric field generated by two castellated electrode arrays placed 1 mm apart. On applying a 16 Volt peak-to-peak sinusoidal voltage to the electrodes, the suspended cells were pulled into the plane of the electrode arrays by the dielectrophoretic force and collected between the interdigitated electrodes. Such re-

moval of cells from the bulk solution was detected as a decrease in absorbance of a light beam passed between the electrode arrays, and the rate of this decrease over 15 s from the time the a.c. field was applied is a measure of the dielectrophoretic force acting on the cells. Full details of the apparatus and experimental technique have been given elsewhere [10,11]. The non-linear electric field generated by 40 μm geometry interdigitated electrodes has also been described [8]. The electrodes employed for this work were of identical form but based on an 80 μm geometry, which was found to be optimal for work with cells of approx. 10 μm diameter. Dielectrophoretic responses were measured for applied a.c. fields over the frequency range 1 Hz to 2 MHz, with five measurements being taken over each decade within this range. The measurement at each frequency was conducted on a fresh aliquot of the cell suspension in order to ensure that the cells remained viable.

Results

MEL cells are transformed precursors of red blood cells that have not differentiated beyond the stage of colony-forming cells [17]. They are well established as a model for the study of cell differentiation because of their susceptibility to a variety of chemical agents that can, in some clones, induce varying degrees of differentiation up to an advanced step of erythroid differentiation that has much in common with the late steps of the normal nuclear extrusion process [20]. Clone DS19 progresses beyond the colony-forming stage to the stage of terminal differentiation in response to treatment with HMBA, evidenced by haemoglobin production and loss of the ability to grow in soft agar. It has been shown [30] for the various cell lines that the average cell diameter decreases by approx. 12% and the cell surface charge density decreases by 14% after treatment with the inducing agent HMBA.

Earlier studies [10,11] have shown that the dielectrophoretic properties of cells can be characterised according to the responses observed in three frequency bands. For frequencies below 200 Hz, the dielectrophoretic response appears to be primarily influenced by the cell surface charge, whilst between 200 Hz to around 1.5 kHz the effective conductivity of the cell is the controlling factor. For frequencies above 1.5 kHz the dielectrophoretic response is determined by the dielectric permittivity of the cell. Eqn. 1 indicates that a change in cell volume alone alters the magnitude of the dielectrophoretic spectrum, but leaves unaltered the relative contributions from surface charge, cell conductivity and permittivity. The results obtained for the various cell lines studied will be described in terms of these three characteristic properties.

The dielectrophoretic response (collection rate) for clone DS19 before, and after, induction by HMBA is shown in Fig. 2. For frequencies below 30 Hz the

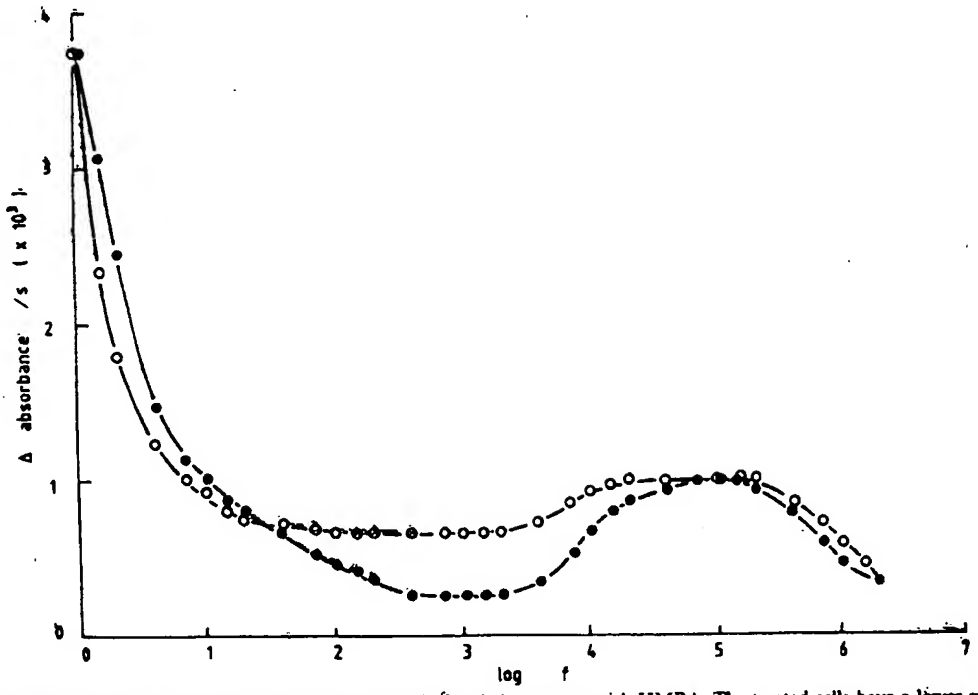

Fig. 2. Dielectrophoretic response of clone DS19 before (●) and after (○) teatment with HMBA. The treated cells have a lower surface charge and larger effective conductivity.

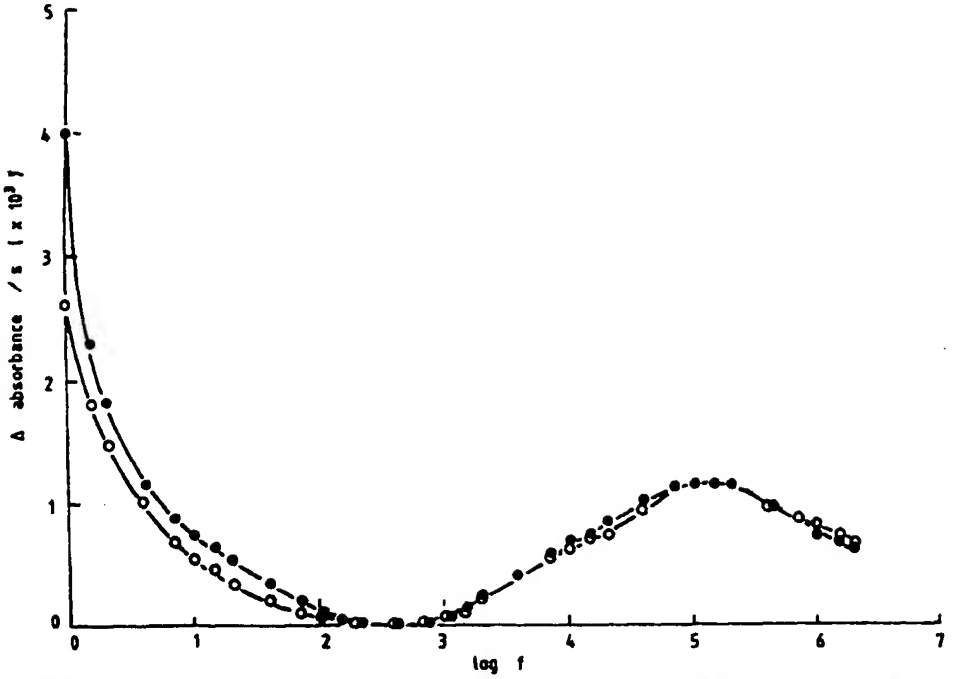

Fig. 3. Dielectrophoretic response of clone R1 before (●) and after (○) treatment with HMBA. The treated cells have a slightly smaller surface charge, but unchanged conductivity and permittivity.

treated cells have a lower dielectrophoretic response than the untreated cells, which, from earlier studies [11], indicates that the treated cells have a reduced surface charge. This result is in agreement with cell micro-electrophoresis data for DS19 [30]. The dielectrophoresis spectrum shows a larger response for the treated cells

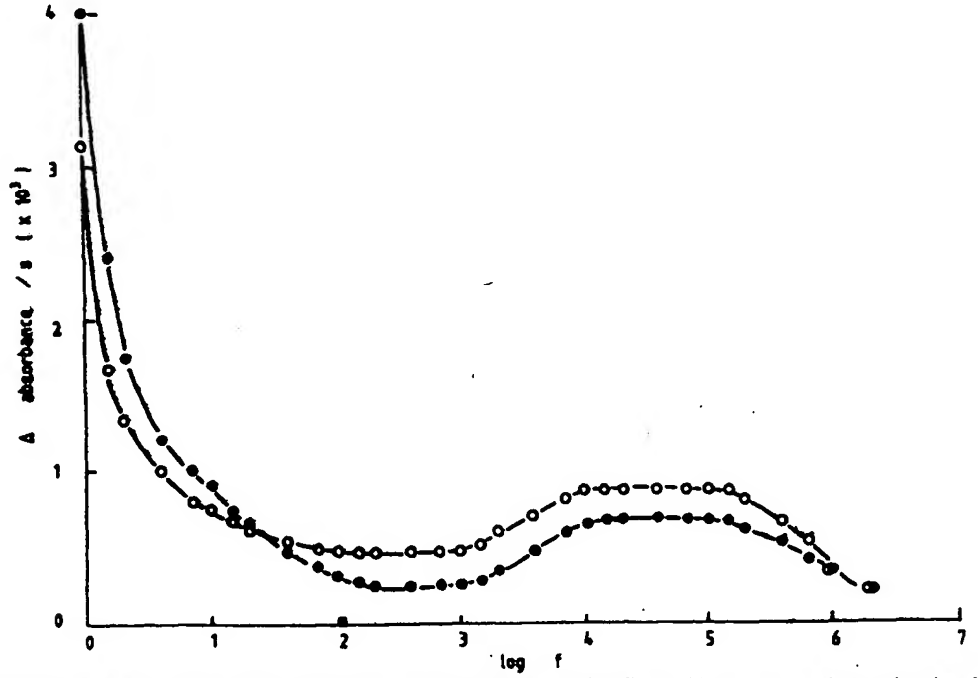

Fig. 4. Dielectrophoretic response of clone DR10 before (●) and after (○) treatment with HMBA. The treated cells have reduced surface charge and increased conductivity and permittivity.

than for untreated cells in the frequency range 30 Hz to 70 kHz, which indicates that as a result of HMBA treatment the effective conductivity of the cells is increased. For frequencies above 70 kHz the collection is independent of treatment indicating that the cell permittivity is unchanged by the HMBA.

The dielectrophoretic responses for clone R1 with and without HMBA treatment are shown in Fig. 3. Unlike DS19, clone R1 does not produce haemoglobin or lose its ability to grow in soft agar after treatment with inducing agents and is therefore classified as being non-inducible [30]. Fig. 3 shows the dielectrophoretic response below 200 Hz to be reduced on HMBA treatment, indicating as in the case of DS19 a reduction in cell surface charge. This result is also consistent with micro-electrophoresis measurements, where clone R1 was found to lose net surface charge in the same way as clone DS19 after exposure to HMBA [30]. For frequencies above 200 Hz the response remains unchanged showing that HMBA treatment has no effect on either cell conductivity or permittivity.

Clone DR10 is characterised by a differential response to the inducing agent HMBA and DMSO. Whereas this clone responds to HMBA treatment by producing haemoglobin and losing its ability to grow in soft agar, it is resistant to DMSO, which is a strong inducing agent for DS19 [30]. The dielectrophoretic responses shown in Fig. 4 show that after treatment with HMBA the DR10 cells lose surface charge, shown by a reduction in the response below 100 Hz, and gain in effective conductivity, shown by an increase in response between 100 Hz and 5 kHz. Above 5 kHz the dielectrophoretic response is also increased, which is consistent with a gain in the permittivity of the DR10 cells with HMBA treatment. As shown in Fig. 5, however, after treatment with DMSO the dielectrophoretic characteristics of the DR10 cells remains unchanged over the whole frequency range, implying that DMSO treatment has no effect on the surface charge, conductivity or permittivity of DR10 cells.

These results indicate that for each case where the cells respond to the inducing agents, a definite change in dielectrophoretic response was observed. However, in the case of R1 treated with HMBA and DR10 treated with DMSO, where no differentiation was induced the dielectrophoretic responses remained unchanged.

To test the contribution of cell surface charge to the dielectrophoretic response, fresh human blood, collected in EDTA, was washed three times in Hanks' buffered saline solution containing 3 mg/ml glucose. The red blood cells were resuspended in 10 ml of this same solution containing 0.25 units of neuraminidase (Sigma n-2133 Type X from *Clostridium perfringens*) and incubated for 40 min at 37°C with an occasional agitation to keep the cells suspended. A parallel sample prepared in the same manner, but without the addition of neuraminidase served as a control. The dielectrophoretic responses of these cell samples were measured

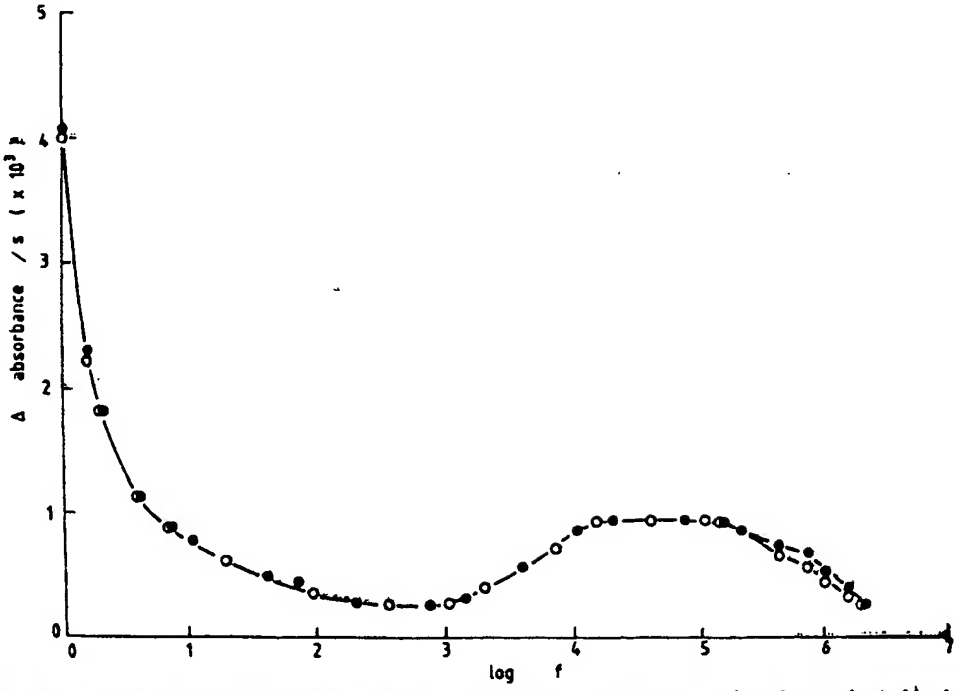

Fig. 5. Dielectrophoretic response of clone DR10 before (●) and after (○) treatment with DMSO, to show that no observable changes occur in the electrical properties of the cells.

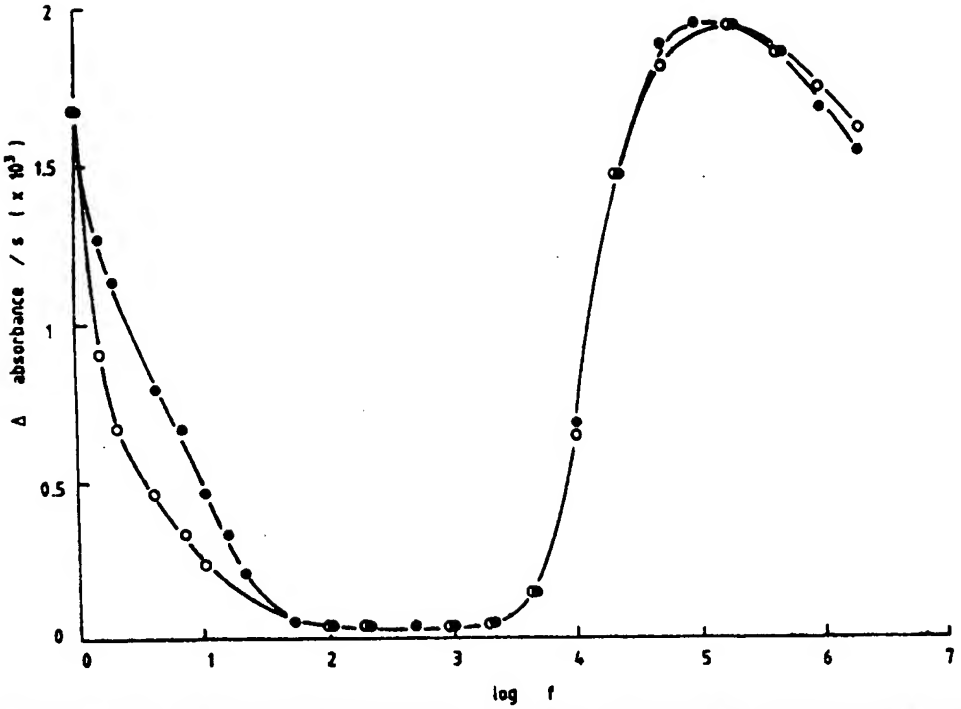

Fig. 6. Dielectrophoretic response of human red blood cells before (●) and after (○) neuraminidase treatment. The treated cells have reduced surface charge.

after they had been washed and resuspended in dielectrophoresis medium in the usual way. Treatment with neuraminidase is a well tested method for reducing charge associated with sialic acid residues on the cell membrane surface. Following the treatment described here the micro-electrophoretic mobility of the blood

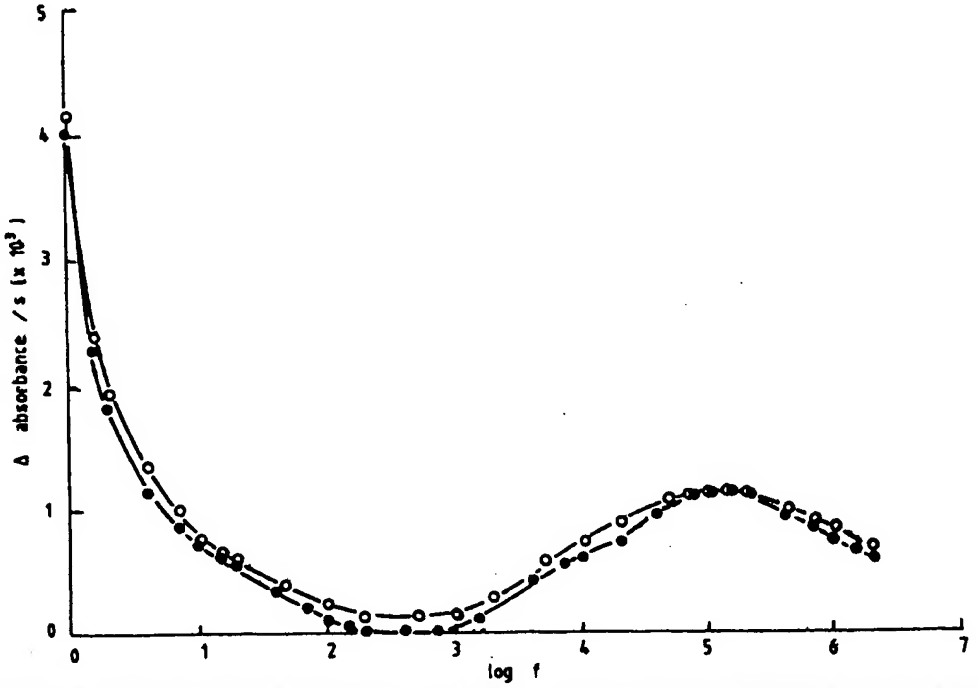

Fig. 7. The dielectrophoretic response of clone R1 cells before (●) and after (○) saponin treatment, indicating that saponin slightly increases the effective cell conductivity.

cells was found to have decreased by 50%, showing that the net cell membrane surface charge had been halved. The spectra of Fig. 6 show that the neuraminidase-treated cells exhibited a clear decrease in dielectrophoretic collection rate below 50 Hz. The unchanged response above 50 Hz demonstrates that the effective conductivity and permittivity of the cell membrane was not changed by this treatment. This result supports the conclusion that cell surface charge influences the dielectrophoretic behaviour only in the low frequency range [10,11].

To verify our previous conclusion [10,11] that membrane conductivity influences the mid-range frequency response, freshly harvested R1 cells were treated with saponin (40 μg/ml) for 5 min. This is a treatment shown [21] to porate the cell membrane and to permeabilise it without causing a major loss of cytoplasmic protein. The cells were then washed and resuspended in the dielectrophoresis medium, and investigated in the same manner as before. The effect of this treatment is shown in Fig. 7, where the effective membrane conductivity appears to be increased as evidenced by the increased dielectrophoretic response in the frequency range 100 Hz to 1.5 kHz, and the cell surface charge and permittivity are left unaltered.

Discussion

The electrical properties of cells, and the physicochemical origins of these properties have recently been reviewed [22]. If we consider, in simple form, a section of a cell membane of area, A, and thickness, d, then its passive electrical properties are completely characterised by the measured electrical capacitance, C, (units Farads) and conductance G (units Ohms^{-1} or Siemens) as defined by

$$G = A\sigma/d\,;\; C = Ae_0e/d$$

The conductivity, σ, is a measure of the ease with which free charge carriers can move in the membrane under the influence of an electric field. Depending on the orientation of the membrane segment with respect to the applied electric field the charge movements can be either predominantly perpendicular or parallel to the external membrane surface. The factor e_0 is the permittivity of free space whilst e is the relative permittivity of the membrane material and is a measure of the extent to which locally bound charge distributions can be distorted or polarised under the influence of an imposed electric field. As discussed elsewhere [22], the effective capacitance of a typical cell membrane is of the order 1 μF/cm^2, and the lateral electrophoresis of charged membrane proteins and lipids appear to be a contributing factor. Electrical double layer effects, associated with hydrated ions that are attracted to the membrane surface by membrane bound charges such as those occurring on lipid head groups, can also give rise to polarisations that add to the effective capacitance. These effects can also contribute to dielectric loss processes, which appear as components of the membrane surface conductance K_s.

Cell membranes, being composed largely of non-conducting lipids and protein material, are not good electrical conductors in the normal sense. At frequencies below approx. 100 kHz, the low value of the bulk membrane conductivity σ_b prevents the applied electric field from penetrating into the cell interior. As the frequency is increased above 100 kHz, the membrane resistance begins to be electrically short-circuited by the membrane capacitance, and the electric field proceeds to penetrate the cell. For frequencies below 100 kHz the overall effective conductivity σ_p of the cell, can be written as [23]

$$\sigma_p = \sigma_b + \frac{2K_s}{r} \qquad (2)$$

where r is the cell radius. In this formula, which has been used with success in investigations of the surface conductance of latex particles [24] and in electrorotation studies of various types of cell [25], the parameter σ_b can be taken to represent the membrane bulk conductivity, since the cell membrane effectively shields the cell interior from the applied electric field [26]. In other words the cell is considered to be a poorly conducting particle of high relative permittivity [26], and σ_b represents the effective conductivity of that region of the membrane penetrated by the applied electric field. As already mentioned, the passive membrane bulk conductance is generally very small and a typical case is the low-frequency (10 Hz) anion conductance value of around 60 mS/m^2 for erythrocyte membranes [27]. For a membrane thickness near 8 nm this gives a membrane conductivity value (σ_b) of about 0.5 nS/m. The largest membrane conductivity value reported appears to be that of 140 S/m^2 for oocytes [28], which for a typical membrane thickness gives a σ_b value of around 1 μS/m. To our knowledge no definitive values have been derived for the surface conductance K_s of animal cells, but of possible relevance is the value of 1 nS obtained for carboxylate latex particles [24]. For a cell radius of 5 μm and a similar K_s value, then the corresponding value for the factor 2 K_s/r in Eqn. 2 is 400 μS/m. The conductivity of the cell suspending solution was of the order 340 μS/m, and for positive dielectrophoresis to be observed the effective conductivity σ_p of the cells must be greater than this. These considerations lead to the conclusion that the most dominant factor influencing the effective conductivity of the cells in our measurements are tangential membrane conduction processes rather than trans-membrane conduction.

The results given in Fig. 6, showing that neuraminidase reduces the dielectrophoretic response of red blood cells between 1 Hz and 100 Hz, but has little effect outside this frequency range, provide support for the conclusion [10,11] that surface charge effects dominate the low frequency dielectrophoretic behaviour of cells. Another significant finding (Fig. 7) is the fact that saponin produces only a small effect on the dielectrophoretic properties of R1 cells. This can be quantified by calculating the change of effective cell conductivity after saponin treatment. The theory for this is detailed elsewhere [8], but basically it can be shown that at low frequencies the polarisability parameter, p, of Eqn. 1 reduces to the expression

$$p = \epsilon_0 \epsilon_m \left(\frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m} \right) \quad (3)$$

where the subscripts p and m refer to the particle and suspending medium, respectively. Calculations based on 1 kHz measurements show that treating DS19 and DR10 cells with HMBA increases their effective conductivity (compared to the increase induced in R1 cells with saponin) by a factor of 3 and 1.67, respectively. (The fact that HMBA reduces the effective cell diameter by just over 10% has not been taken into account using Eqn. 1, but inspection of Eqn. 2 indicates that there is a real conductivity increase.) Since saponin treatment is known [20] to depolarise cells and even to produce holes in the membrane large enough for macromolecules to escape, it would be expected to increase the trans-membrane conductivity much more than HMBA treatment, which allows the trans-membrane potential to be maintained. By comparing the results of Figs. 2 and 6 it is seen that saponin produced a smaller change in effective membrane conductivity in R1 cells than that produced by HMBA treatment in DS19 cells. This would imply, for R1 cells at least, that the effective conductivity as measured by dielectrophoresis is largely controlled by tangential surface conductivity processes, rather than by trans-membrane charge transport. This confirms our conclusions above concerning the relative magnitudes of σ_b and K_s in Eqn. 2.

The results of Figs. 2 and 3, showing that HMBA decreases the low-frequency (1 Hz to 200 Hz) dielectrophoretic response (and hence the surface charge) of the inducible DS19 cells as well as the non-inducible R1 cells, agrees with the earlier electrophoresis work [30]. The interesting and new finding is that from Fig. 2, HMBA increases the mid-frequency (200 Hz to 10 kHz) dielectrophoretic response (and hence the effective cell conductivity) of the inducible DS19 cells, but does not influence the effective conductivity of the non-inducible R1 cells (Fig. 3). This is mirrored in the results of Figs. 4 and 5, which show that the effective conductivity of DR10 cells is increased by HMBA treatment, but is not altered by DMSO treatment. Cell induction thus appears to be accompanied by an increase of the effective cell conductivity, and since HMBA does not induce cell membrane depolarisation (unlike saponin which does) this effect cannot arise from an enhancement of the trans-membrane conductivity. Furthermore, HMBA decreases the cell surface charge (Ref. 30 and Figs. 2–5), which might be expected to lead to a reduction of polarisations associated with the presence of hydrated counter-ions located near surface bound charges. These various factors again emphasise that the lateral, rather than the trans-membrane, conductivity component is the most dominant parameter influencing the effective cell conductivity. An obvious concept to invoke here is the possibility that the lateral electrophoretic mobility of charged membrane proteins and lipids is enhanced through an increase of membrane fluidity. However, in MEL cells it is known [29] that HMBA treatment reduces membrane fluidity. This suggests that the dominant lateral conductivity component might be related to delocalised membrane-associated ions (or protons), or polarisable fixed dipole charge distributions, changes of which could be connected to the membrane lipid compositional alterations that occur during MEL cell induction.

Conclusions

Three lines (DS19, R1 and DR10) of Friend murine erythroleukaemic cells have been studied by dielectrophoretic measurement, as a function of treatment with HMBA. Human red blood cells have also been investigated as a function of neuraminidase treatment, and the results obtained confirm that the low frequency dielectrophoretic response is controlled by cell surface charge effects. On the basis of this, the dielectrophoretic responses observed for DS19, R1 and DR10 cells can be interpreted to show that the surface charge of the cells becomes less negative on treatment with HMBA, which is a result in agreement with previous electrophoresis measurements [30]. Although HMBA reduces the surface charge of DS19, R1 and DR10, only DS19 and DR10 are induced to differentiate. A new finding obtained from these dielectrophoresis measurements is that on induction with HMBA the effective conductivity of the DS19 and DR10 cells is increased. This increase in conductivity is considered to be related to an increase in either the lateral electrophoretic mobility of delocalised ions or the polarisability of dipoles at the surface of the cell membranes. The conclusion concerning the dominance of tangential membrane conductivity, over that of trans-membrane conductivity, is given support by the finding that saponin treatment has only a small influence on the dielectrophoretic response in the mid-frequency region (100 Hz to 1.5 kHz) which is controlled by membrane conductivity effects. This result is

also consistent with the assessment of Eqn. 2 in terms of the present (albeit limited) knowledge of the characteristic membrane conductivity parameters for cells.

Dielectrophoresis, the study of the motion of particles in non-uniform a.c. electric fields, has not been widely employed in biological research. The development of a simple, low-cost, optical technique as used in these studies provides a new method for investigating and distinguishing between cell surface charge and cell membrane conductivity properties as a function of cell physiology.

Acknowledgements

This work is supported by the National Foundation for Cancer Research (U.S.A.). We thank Robyn Rhea and Jamileh Noshari for their work in maintaining and characterising the cell lines.

References

1 Pohl, H.A. (1978) Dielectrophoresis, Cambridge University Press, Cambridge.
2 Lin, I.J. and Benguigui, L. (1982) J. Electrostatics 13, 257–262.
3 Jones, T.B. and Kraybill, J.P. (1986) J. Appl. Phys. 60, 1247–1252.
4 Stoicheva, N. and Dimitrov, D.S. (1986) Electrophoresis 7, 339–341.
5 Dimitrov, D.S. and Zhelev, D.V. (1987) Bioelectrochemistry Bioenerg. 17, 549–557.
6 Pohl, H.A. and Pethig, R. (1977) J. Phys. E: Sci. Instrum. 10, 190–193.
7 Feely, C.M. and Pohl, H.A. (1981) J. Phys. D: Appl. Phys. 14, 2129–2138.
8 Price, J.A.R., Burt, J.P.H. and Pethig, R. (1988) Biochim. Biophys. Acta 964, 221–230.
9 Inoue, T., Pethig, R., Al-Ameen, T.A.K., Burt, J.P.H. and Price, J.A.R. (1988) J. Electrostatics 13, 257–262.
10 Burt, J.P.H., Al-Ameen, T.A.K. and Pethig, R. (1989) J. Phys. E: Sci. Instrum. 22, 952–957.
11 Burt, J.P.H. and Pethig, R. (1989) in Electric Field Phenomena in Biological Systems (Paris, R., ed.), Inst. of Phys. Short Meeting Series No. 21, 15–26.
12 Bingelli, R. and Cameron, I.V. (1980) Cancer Res. 40, 1830–1845.
13 Boonstra, J., Mummery, C.L., Tertoolen, L.G.J., Van Der Saag, P.T. and DeLaat, S.W. (1981) J. Cell. Physiol. 107, 75–83.
14 Cone, C.D. (1974) Ann. NY Acad. Sci. 238, 420–435.
15 Lai, C.-N., Gallick, G.E., Arlinghaus, R.B. and Becker, F.F. (1984) J. Cell Physiol. 121, 139–142.
16 Price, J.A.R., Pethig, R., Lai, C.-N., Becker, F.F., Gascoyne, P.R.C. and Szent-Györgyi, A. (1987) Biochim. Biophys. Acta 898, 129–136.
17 Marks, P.A., Sheffery, M. and Rifkind, R.A. (1987) Cancer Res. 47, 659–666.
18 Bashford, C.L., Alder, G.M., Graham, J.M., Mestrina, O. and Pasternak, C.A. (1988) J. Membr. Biol. 103, 79–94.
19 Pasternak, C.A. (1988) Biosci. Rep. 8, 579–583.
20 Malik, Z., Lugaci, H. and Hanania, J. (1988) J. Exp. Hematol. 16, 330–335.
21 Wassler, M., Jonasson, I., Persson, R. and Fries, E. (1987) Biochem. J. 247, 407–415.
22 Pethig, R. and Kell, D.B. (1987) Phys. Med. Biol. 32, 933–970.
23 Schwan, H.P., Schwarz, G., Maczuk, J. and Pauly, H. (1962) J. Phys. Chem. 66, 2626–2635.
24 Arnold, W.M., Schwan, H.P. and Zimmermann, U. (1987) J. Phys. Chem. 91, 5093–5098.
25 Arnold, W.M. and Zimmermann, U. (1988) J. Electrostatics 21, 151–191.
26 Schwan, H.P. (1988) Ferroelectrics 86, 205–223.
27 Takashima, S., Asami, K. and Takahashi, Y. (1988) Biophys. J. 54, 995–1000.
28 Arnold, W.M., Schmutzler, R.K., Al-Hasani, S., Krebs, D. and Zimmermann, U. (1989) Biochim. Biophys. Acta 979, 142–146.
29 Ip, S.H.C. and Cooper, R.A. (1980) Blood 56, 227–232.
30 Gascoyne, P.R.C. and Becker, F.F. (1990) J. Cell. Physiol. 142, 309–315.